U.S. Patent Application No. 09/706,243

REGULATION OF ENDOGENOUS GENE EXPRESSION

IN CELLS USING ZINC FINGER PROTEINS

Pending Claims: October 2001

118. A method of inhibiting expression of an endogenous cellular gene in a cell, the method comprising the step of:

contacting a first target site in the endogenous cellular gene with an engineered zinc finger protein, wherein the K_d of the zinc finger protein is less than about 25 nM;

thereby inhibiting expression of the endogenous cellular gene.

119. The method of claim 118, wherein the step of contacting further comprises contacting a second target site in the endogenous cellular gene with a second zinc finger protein.

120. The method of claim 119, wherein the first and second target sites are adjacent.

121. The method of claim 120, wherein the first and second zinc finger proteins are covalently linked to form a fusion protein.

122. The method of claim 118, wherein the first zinc finger protein is a fusion protein comprising a regulatory domain.

123. The method of claim 122, wherein the first zinc finger protein is a fusion protein comprising at least two regulatory domains.

124. The method of claim 119, wherein the first and second zinc finger proteins are fusion proteins, each comprising a regulatory domain.

125. The method of claim 124, wherein the first and second zinc finger protein are fusion proteins, each comprising at least two regulatory domains.

126. A method of inhibiting expression of an endogenous cellular gene in a cell, the method comprising the step of:

contacting a target site in the endogenous cellular gene with an engineered fusion zinc finger protein comprising six fingers and a regulatory domain, wherein the K_d of the zinc finger protein is less than about 25 nM;

thereby inhibiting expression of the endogenous cellular gene.

127. The method of claim 118, wherein the cell is selected from the group consisting of an animal cell, a plant cell, a bacterial cell, a protozoal cell, and a fungal cell.

128. The method of claim 127, wherein the cell is a mammalian cell.

129. The method of claim 128, wherein the cell is a human cell.

130. The method of claim 118, wherein expression of the endogenous cellular gene is inhibited by at least about 20%.

131. The method of claim 118, wherein the endogenous cellular gene is selected from the group consisting of VEGF, ERα, IGF-I, c-myc, c-myb, ICAM, and Her2/Neu.

132. The method of claim 131, wherein the endogenous cellular gene is VEGF.

133. The method of claim 118, wherein the inhibition of gene expression prevents gene activation.

134. The method of claim 122, wherein the regulatory domain is selected from the group consisting of a transcriptional repressor, an endonuclease, a methyl transferase, and a histone deacetylase.

135. The method of claim 124, wherein the regulatory domain is selected from the group consisting of a transcriptional repressor, an endonuclease, a methyl transferase, and a histone deacetylase.

136. The method of claim 118, wherein the method further comprises the step of first administering to the cell a delivery vehicle comprising the zinc finger protein, wherein the delivery vehicle comprises a liposome or a membrane translocation polypeptide.

137. The method of claim 118, wherein the target site is upstream of a transcription initiation site of the endogenous cellular gene.

138. The method of claim 118, wherein the target site is adjacent to a transcription initiation site of the endogenous cellular gene.

139. The method of claim 118, wherein the zinc finger protein comprises an SP-1 backbone.

140. The method of claim 139, wherein the zinc finger protein comprises a regulatory domain and is humanized.

141. A method of activating expression of an endogenous cellular gene, the method comprising the step of:

contacting a first target site in the endogenous cellular gene with an engineered zinc finger protein, wherein the K_d of the zinc finger protein is less than about 25 nM;

thereby activating expression of the endogenous cellular gene.

142. The method of claim 141, wherein the step of contacting further comprises contacting a second target site in the endogenous cellular gene with a second zinc finger protein.

143. The method of claim 142, wherein the first and second target sites are adjacent.

144. The method of claim 143, wherein the first and second zinc finger proteins are covalently linked to form a fusion protein.

145. The method of claim 141, wherein the first zinc finger protein is a fusion protein comprising a regulatory domain.

146. The method of claim 145, wherein the first zinc finger protein is a fusion protein comprising at least two regulatory domains.

147. The method of claim 142, wherein the first and second zinc finger proteins are fusion proteins, each comprising a regulatory domain.

148. The method of claim 147, wherein the first and the second zinc finger protein are fusion proteins, each comprising at least two regulatory domains.

149. A method of activating expression of an endogenous cellular gene, the method comprising the step of:

contacting a target site in the endogenous cellular gene with an engineered fusion zinc finger protein comprising six fingers and a regulatory domain, wherein the K_d of the zinc finger protein is less than about 25 nM;

thereby activating expression of the endogenous cellular gene.

150. The method of claim 141, wherein the cell is selected from the group consisting of an animal cell, a plant cell, a bacterial cell, a protozoal cell, and a fungal cell.

151. The method of claim 150, wherein the cell is a mammalian cell.

152. The method of claim 151, wherein the cell is a human cell.

153. The method of claim 141, wherein expression of the endogenous cellular gene is activated to at least about 150%.

154. The method of claim 141, wherein the endogenous cellular gene is selected from the group consisting of FAD2-1, EPO, GM-CSF, GDNF, VEGF, and LDL-R.

155. The method of claim 154, wherein the endogenous cellular gene is VEGF.

156. The method of claim 141, wherein the activation of gene expression prevents repression of gene expression.

157. The method of claim 145, wherein the regulatory domain is selected from the group consisting of a transcriptional activator and a histone acetyltransferase.

158. The method of claim 147, wherein the regulatory domain is selected from the group consisting of a transcriptional activator and a histone acetyltransferase.

159. The method of claim 141, wherein the target site is upstream of a transcription initiation site of the endogenous cellular gene.

160. The method of claim 141, wherein the target site is adjacent to a transcription initiation site of the endogenous cellular gene.

161. The method of claim 141, wherein the zinc finger protein comprises an SP-1 backbone.

162 The method of claim 161, wherein the zinc finger protein comprises a regulatory domain and is humanized.

163. A method of modulating expression of an endogenous cellular gene in a cell, the method comprising the step of:

contacting a first target site in the endogenous cellular gene with an engineered zinc finger protein;

thereby modulating expression of the endogenous cellular gene.

164. The method of claim 163, wherein the step of contacting further comprises contacting a second target site in the endogenous cellular gene with a second zinc finger protein.

165. The method of claim 164, wherein the first and second target sites are adjacent.

166. The method of claim 165, wherein the first and second zinc finger proteins are covalently linked to form a fusion protein.

167. The method of claim 163, wherein the first zinc finger protein is a fusion protein comprising a regulatory domain.

168. The method of claim 167, wherein the first zinc finger protein is a fusion protein comprising at least two regulatory domains.

169. The method of claim 164, wherein the first and second zinc finger proteins are fusion proteins, each comprising a regulatory domain.

170. The method of claim 169, wherein the first and second zinc finger protein are fusion proteins, each comprising at least two regulatory domains.

171. A method of modulating expression of an endogenous cellular gene in a cell, the method comprising the step of:

contacting a target site in the endogenous cellular gene with an engineered fusion zinc finger protein comprising six fingers and a regulatory domain;

thereby modulating expression of the endogenous cellular gene.

172. The method of claim 163, wherein the cell is selected from the group consisting of an animal cell, a plant cell, a bacterial cell, a protozoal cell, and a fungal cell.

173. The method of claim 172, wherein the cell is a mammalian cell.

174. The method of claim 173, wherein the cell is a human cell.

175. The method of claim 163, wherein the endogenous cellular gene is selected from the group consisting of VEGF, ERα, IGF-I, c-myc, c-myb, ICAM, Her2/Neu, FAD2-1, EPO, GM-CSF, GDNF, and LDL-R.

176. The method of claim 175, wherein the endogenous cellular gene is VEGF.

177. The method of claim 167, wherein the regulatory domain is selected from the group consisting of a transcriptional repressor, a transcriptional activator, an endonuclease, a methyl transferase, a histone acetyltransferase, and a histone deacetylase.

178. The method of claim 169, wherein the regulatory domain is selected from the group consisting of a transcriptional repressor, a transcriptional activator, an endonuclease, a methyl transferase, a histone acetyltransferase, and a histone deacetylase.

179. The method of claim 163, wherein the method further comprises the step of first administering to the cell a delivery vehicle comprising the zinc finger protein, wherein the delivery vehicle comprises a liposome or a membrane translocation polypeptide.

180. The method of claim 163, wherein the target site is upstream of a transcription initiation site of the endogenous cellular gene.

181. The method of claim 163, wherein the target site is adjacent to a transcription initiation site of the endogenous cellular gene.

182. The method of claim 163, wherein the zinc finger protein comprises an SP-1 backbone.

183. The method of claim 182, wherein the zinc finger protein comprises a regulatory domain and is humanized.